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Extraction and Evaluation of Pharmacological Activity of Pigments from Purple Cam (*Peristrophe bivalvis* (L.) Merr)

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ABSTRACT

Objectives: Acquiring pigment extract to evaluate the antioxidant and toxic activity for human lung and liver cancer cell lines.
Method: Take Purple Cam stems and leaves to boil in distilled water; spin the vacuum to obtain a high pigment. This extract tested antioxidant activity, anti-inflammatory activity, and cytotoxic activity of A549 lung and Hep3B liver cancer in humans.
Results: extracts from the leaves and purple mulberry leaves with an extraction efficiency of 9.16%, the pigment extract obtained with antioxidant activity at the test concentration of 500µg/ml had a percentage of inhibition (% Inhibition) reached 71.04. Both the test concentrations of 30 µg/ml and 100 µg/ml, the pigment of rosemary, have no anti-inflammatory activity but have the ability to kill human lung cancer cells A549 and Hep3B human liver at levels average.
Conclusion: The extract of Purple Cam does not show anti-inflammatory activity, but it is capable of killing human lung cancer cell and liver cancer.

Keywords: Broccoli, antioxidant, anti-inflammatory, pigment, cancer

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INTRODUCTION

In the current situation, the number of people suffering from food poisoning due to the abuse of synthetic color is increasing, causing adverse effects on human health. Therefore, the general trend of the world is to find and extract natural pigments that can be used in the food industry from plant materials or semi-synthetic [1].

Broccoli is one of the annual herbaceous plants with many applications such as medicinal herbs and plant pigments [1]. This plant is very popular in many countries such as Cambodia, China, India, Indonesia, Laos, Malaysia, Thailand, and Vietnam [2]. For a long time, purple rosewood leaf extract has been used as a food colorant in Vietnam and many countries in Asia [3]. A number of studies have shown that the extract from the leaves is beneficial for human health [4], [5], [6].

In Vietnam, plants are abundant in Muong Khuong (Lao Cai), Moc Chau (Son La), and Thai Nguyen [1]. Authors Do Thi Xuyen and Nguyen Thi Phuong Thao (2007) distinguished the ecological characteristics between rosewood and periwinkle (Dicliptera Chinensis (L.) Juss.) [7]. The research team of author Jiang et al. (2011) obtained red pigments from rosewood by extraction technique combining ultrasonic waves with an alcohol-based solvent [8]. Several other studies have also shown that the leaves extract has a strong resistance to microbial activity such as Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli [5], [9].

The objective of the study is to fully describe the morphological and anatomical characteristics of collected Purple Cam in Vietnam; extract and obtain high pigments from stems and leaves for long-term use and preservation of pigments; Assessment of biochemical activity of hyperpigmentation (anti-oxidant, anti-inflammatory, killing lung cancer and human liver cancer).

Materials and methods

Subject, time and place of study: The Purple Cam collected from Vietnam is used as the raw material for research experiments. The study was conducted at the Plant Physiology Laboratory, the Physical Chemistry Laboratory (Hanoi University of Education 2) and the Research Center for Anthropology and Intelligence Development (National University of Education). Hanoi). Experiments to test antioxidant, anti-inflammatory and toxic effects on human liver and lung cancer cell lines were conducted at the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology.

Chemicals include carmin dye, javen solution (NaClO) (China), methylene blue (China), distilled water. Tools: slide glass, razor, lamen, suction tube, filter paper, petri dish, watch plate

Equipment: microscopes (Carl Zeiss), ovens (Memmert), technical scales (Satorious), microwaves (LG), refrigerators (Toshiba), rotary vacuum evaporator Strike 300 (Steroglass).

Method: Observing and describing plant, leaf and flower morphology in specialized terms. The method of making temporary templates to study the anatomical structure of leaves, petioles, stems [10]. The experimental method of obtaining pigment from Purple Cam stems and leaves. Take purple Cam's stem and leaves (300 grams) and boil in 500 ml of distilled water for 15 minutes; vacuum distillation to obtain the extract.

Test method of biochemical activity of the extract: The Purple Cam tree extracts were tested for antioxidant activity using 1,1-Diphenyl 1-2 picrylhydrazyl (DPPH) as substrate; Anti-inflammatory activity test using MTT (3-(4,5-dimethythiazol-2-yl) -2,5-diphenyl tetrazolium bromide) as a substrate on RAW264.7 cell line; the test of cytotoxic activity of human lung cancer cell A549 and human liver cancer Hep3B. These tests were conducted by

the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology.

Result and discussion

Morphological and anatomical characteristics of purple Cam

According to the survey of author Nguyen Thi Phuong Thao et al (2009), species (Peristrophe bivalvis (L.) Merr., Syn. P. roxburghiana (Schult.) Bremex.) In Muong Khuong, Lao Cai includes 4 types (Red Cam; 02 forms of Purple Cam; 1 form of yellow Cam) [1]. In this study, a sample of Purple Cam was collected in Vietnam. The morphological and anatomical results are shown in Figure 1

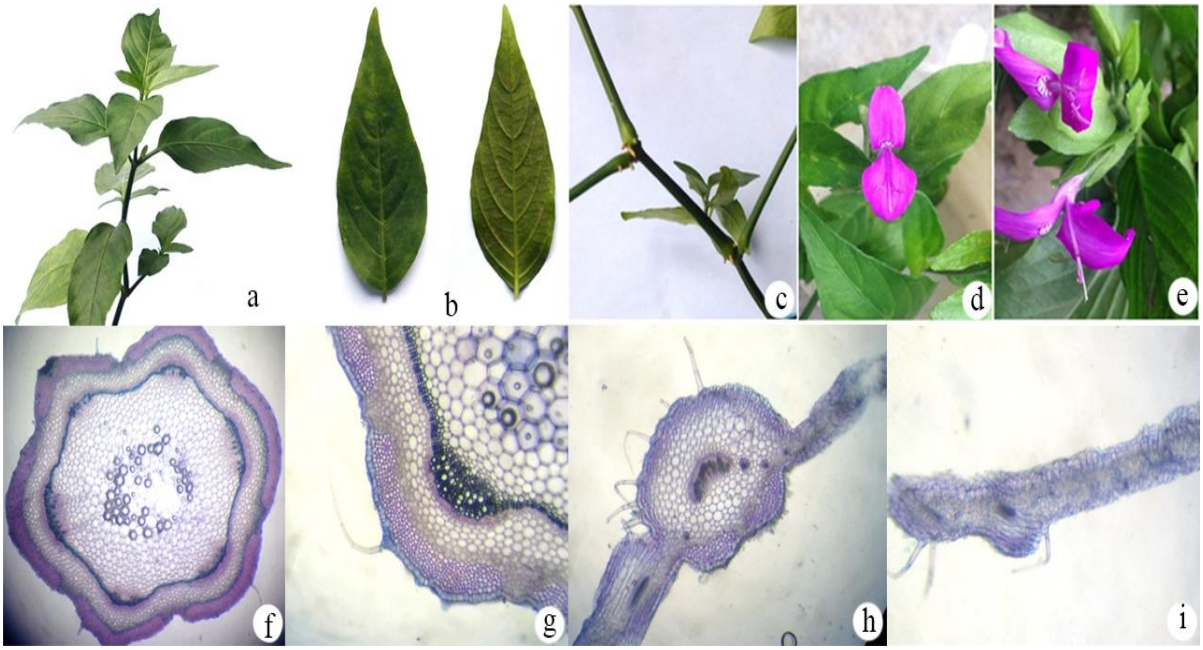

Fig 1. Morphological-anatomical characteristics of purple Cam

a. Bough; b. The top and bottom of the leaf; c. The stem segment has roots; d, e. Flower; f, g. The template crosses the body, h, i. The template crosses the leaf stalk and leaf blade

The results showed that the Purple Cam tree has a herbaceous form of 30-60 cm tall, on the stem with secondary roots (Figure 1 a, d), clear leaves and petioles, with a length of 3-4 cm, leaf blades with pointed edged lanceolate (Figure 3.1 b, c), wood vein and secondary libe, conjoined petals in tubular shape on the lower part, upper divided into two lips (Figure 1 e, f). In this study, purple camellia has morphological and anatomical characteristics similar to those described by Nguyen Thi Phuong Thao et al. (2009) [1]; Do Thi Xuyen and Nguyen Thi Phuong Thao (2007) [6]. According to the "List of Vietnamese Plant Species" [11], the Peristrophe Nees genus has 4 species, of which only Cam (P. bivalvis (L.) Merr.) In the North.

The process of extracting and acquiring extracts from purple Cam

According to the research of the author Nguyen Thi Phuong Thao et al. (2009), the leaves were hot and cold extracted. The extract is filtered through a coarse cloth filter. The filtrate is concentrated on a water bath to a soft, high form, the temperature of the extract when she is in the range of 60-65oC to obtain a high softness [1]. In this study, purple camellia stems and leaves were boiled with distilled water, then the pigment was obtained by vacuum rotary distillation; conduct experiments on dyeing glutinous rice to prove its high pigment properties. The result is shown in Figure 2.

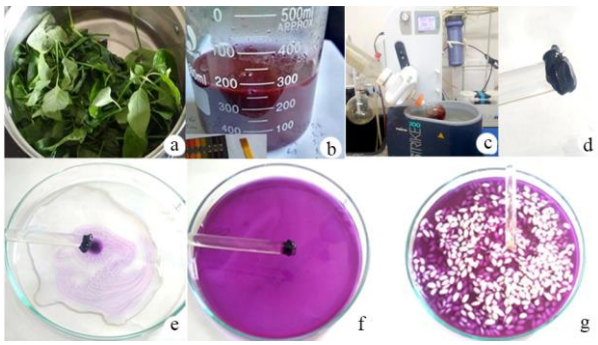

Fig 2. The process of extracting, acquiring and proving the properties of the extract from the purple Cam

a. Fresh stems and leaves are boiled in distilled water for 15 minutes, b. The resulting pigment water (400 ml) has a pH = 6; c. Vacuum distilled water pigment; d. Highly pigmented; e, f, g. High solubility in water and experimental dyeing of glutinous rice From 300 grams of fresh stems and leaves, initially boiled in 500 ml of distilled water 2 times, obtained 400 ml of water pigment, after vacuum distillation, obtained 27.5 grams of high pigmentation (yield reached 9.16 %) (Figure 2 a, b, c, d). The resulting pigment is similar to that extracted from the leaves of fresh Purple Cam leaves (Figure 3.2e, f, g). The efficiency of extracting pigment from purple stems is 1.58%, from purple leaves is 4.98% (compared to fresh weight). In our study, the extract was extracted by vacuum, the extraction efficiency reached 9.16%, the efficiency in this process was 1.83 times higher than the research results of the author Nguyen Thi Phuong Thao. et al. (2009) [1].

The biochemical test of the pigment extract from the purple Cam

Antioxidant test

In this study, tested the antioxidant activity of the pigment extract from the purple Cam. The results are shown in Table 1.

Table 1. Results of antioxidant activity test

Sample	Concentration test (µg/ml)	inhibition rate (%)
Pigment extract	100	11,91 ± 1,61
	500	71,04 ±1,59
Ascorbic acid*	10	15,09 ± 1,08
	50	93,33 ± 0,16

**Ascorbic acid: is used as a positive standard to compare method stability*

The results of Table 1 show that the pigment extracted by water from Purple Cam stems and leaves has good DPPH free radical scavenging activity at a test concentration of 500 µg / ml, % inhibition reaches 71.04% (sample contains only muscle. DPPH substance has a% inhibition of 0). This result is also consistent with some recent studies suggesting that Zizilan compound in marijuana leaves has the ability to treat obesity, hepatitis B, antioxidants, improve the immune system, or use it as a dye. color in many areas [12].

Testing anti-inflammatory activity of the pigment *extract* from the purple Cam

Uncontrolled inflammation may be a factor in chronic diseases. During the inflammatory process, inflammatory cells (neutrophils, eosinophils, monocytes and macrophages) are activated to release large amounts of nitric oxide (NO), prostaglandin E2 (PGE2) and other Pre-inflammatory cytokines such as IL-1β, IL-6, TNF-α aim to destroy or inhibit the growth of invading microorganisms or cancerous tissue. In this study, the anti-inflammatory activity of hyperpigmentation was assessed on the RAW264.7 cell line. The results shown in Table 3.2 show that at the two test concentrations of 30 µM and 100 µM, samples of leaves extracted from leaves of leaves have no activity to inhibit the production of NO on RAW264.7 cells.

Table 2. The results of screening activity to inhibit the production of nitric oxide (NO) of the sample on RAW264.7 cell line

Sample	Concentration test (µM)	inhibition rate	Error	Live cell rate (%)	Error
Control	-	100,00	0,06	96,03	1,60
LPS	-	0,00	0,99	100,00	1,62
Pigment extract	30	-	-	101,19	0,50
	100	-	-	94,00	2,69
Cardamonin*	0.3	25,63	0,67	101,38	1,48
	3	77,69	0,93	90,15	2,55

**Cardamonin is used as a control*

Testing the ability to kill cell lines of lung and liver cancer in humans

The research results of author Tanavade et al. (2012) showed that the extract from the Purple Cam was resistant to cancer cells [4]. In this study, the pigmented extract showed the ability to kill human lung cancer cell A549 and human liver cancer Hep3B at a test concentration of 100 µg / ml at an average level (shown in Table 3).

Table 3. Results of screening for hepatotoxicity activity Hep 3B and lung cancer A549 of the sample

Sample	Concentration test (µg/ml)	Live cell rate (%)			
		A549		Hep3B	
		Live cell rate (%)	Error	Live cell rate (%)	Error
Control	-	100,00	1,48	100,00	0,79
Pigment extract	30	82,24	1,37	84,42	0,53
	100	61,52	0,43	65,74	1,86
Camptothecin*	0,1	55,66	2,49	54,27	2,01
	10	35,74	0,77	22,64	0,67

** Camptothecin is used as a positive standard*

Conclusion

Describe fully the morphological and anatomical characteristics of the Purple Cam stems and leaves in Vietnam. Completing the process of extracting and obtaining the pigment from purple Cam; retains the same properties with the extract of fresh purple Cam. Pigment extract from Purple Cam pot exhibits oxidative activity with 71% inhibition%, does not show anti-inflammatory activity; At a test concentration of 100 µg / ml, it is capable of killing human lung cancer cell line A549 and Hep3B liver cancer at the average level. Because the antioxidant and anticancer activity is quite promising so hope to find chemical constituents present in pigment in future study.

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Author contributions

First author conceived the idea. All the authors have carried out the research work under the supervision of the

first author. The first author drafted the manuscript.

Conflict of interests

The authors declare no conflicts of interest

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